The Development of the Sperm Duct, Oviduct, and Spermatheca in Tubifex rivulorum.

By

J. Bronté Gatenby,
Exhibitioner of Jesus College, Oxford.

With Plate 24, and a Text-figure.

Introduction.

Some months ago Mr. E. S. Goodrich kindly suggested that I should investigate the developmental history of the sperm duct in Tubifex. In this paper I have also described the formation of the oviduct and spermatheca. There is no modern description of the organogeny of the genital ducts of any Oligochæte of the Tubificid type. In 1886 Bergh (1) described, by means of the serial section method, the development of the genitalia of some Lumbricid worms; but histological detail of the kind, made possible by modern instruments and technique, is naturally lacking. Nevertheless, Bergh's results are, in the main, quite in agreement with my own. When Bergh wrote his paper comparative anatomists believed that the genital ducts of Annelids were always, in some way or other, connected phylogenetically with the nephridia. Even as late as 1895, F. E. Beddard (2), in his admirable monograph on the Oligochæta, wrote: "The generative ducts of the Oligochæta have for a long time been believed to have some connection with the nephridia, but the precise nature of this relation has only quite recently been cleared up." The belief in the phylogenetic relation between nephridium and gonoduct was at that time one of the important doctrines of the comparative anatomist, being applied to the Hirudinea and Polychæta as well; but was soon to be completely upset by Mr. E. S. Goodrich (**3** and **3a**) in a series of important papers published in the 'Quarterly Journal of Microscopical Science.' The main results derived from a study of the genital duct and nephridium in the Polychæta are summed up in a paper published in 1900 (**3***b*). Though Bergh nearly thirty years ago contributed evidence for the modern view of the nature and phylogenetic significance of the gonoduct and nephridium of Oligochæta, a view applying to all Annelida, and showed how completely independent were these structures in Lumbricus, investigators preferred almost to ignore his work, and to search in other types of Oligochæta for evidence of the supposed phylogenetic relation of genital duct and nephridium. Thus, though the modern view of the relation of these organs has been worked out first in the Polychæta, it will be seen that some evidence for Mr. Goodrich's views had already been accumulated in Oligochæta.

It is my pleasant duty to acknowledge my indebtedness to Mr. Goodrich both for valuable preliminary advice and for his kind interest and criticism throughout the work.

Material and Methods.

The worms were washed clear from their tubes, and then placed in a dish of running water, where they were left for twelve to twenty-four hours, till the gut became clear of all grit. After anæsthetising with a few drops of cocaine solution, the worms were killed in the following manner, which I devised after several other methods were tried: An anæsthetised worm was seized by the tail end and laid out straight on a piece of glass plate. Two narrow slips of glass were now apposéd one at each side of the worm, so close as just to touch its sides, but not close enough to compress the animal in any way. A drop of the fixing fluid was placed at one end of the groove in which the worm was confined, and soon was drawn over and around the specimen. Several such plates and strips of glass were used, so that while one worm was being killed others were being prepared. After the fluid had penetrated sufficiently to preclude further torsion, the worm was removed from the slip of glass and thrown into a capsule of fixative until it was properly preserved. I tried several fixatives; but the most satisfactory and uniform results were derived by using a mixture of picro-nitric and corrosive-acetic in equal parts. This mixture was recommended by Mr. Goodrich. One precaution alone should be taken—it is to thoroughly wash the worms in several changes of 70 per cent. alcohol in order to remove as much of the nitric acid as possible. If this is not done, one has a difficulty in inducing the sections to take up picric acid plasma stains and orange G, though eosin is easily used if the sections are soaked in it long enough. Picro-formol-acetic I did not find good. The usual carmine and hæmotoxylin stains were used; but some of my sections were stained in methyl-blue eosin, which was found very useful for distinguishing between and "picking out" the various tissues.

The Order of Development of the Genital Organs in Tubifex.

The gonads are developed very early in Tubifex, and in the smallest worms I have sectioned they are present; but long after they are formed from the cœlomic epithelium of the septa 9/10 and 10/11, the worm grows without any further addition to its genitalia. It is not till the Tubifex is nearly half grown that the other parts of the genital organs begin to appear, and as will be shown below, it is not till some of the genital products are almost ripe that the ducts are fully formed. Bergh also found in Lumbricus that the ducts did not appear till long after the embryonic period.

The funnel of the sperm duct is the first part of the genital ducts to appear. The duct itself next begins to differentiate while the funnel is undergoing further development. When the sperm duct is still incomplete and very rudimentary, the spermatheca begins to appear in segment ten. Last of all, the oviduct is developed. The more highly differentiated structures begin to develop first, while the oviduct, which is very simple, appears quite late.

The Development of the Sperm Duct.

As is well known, the funnel is attached to septum 10/11, and projects into segment ten. The genital opening is situated just above the ventral bundle of setæ of segment eleven.

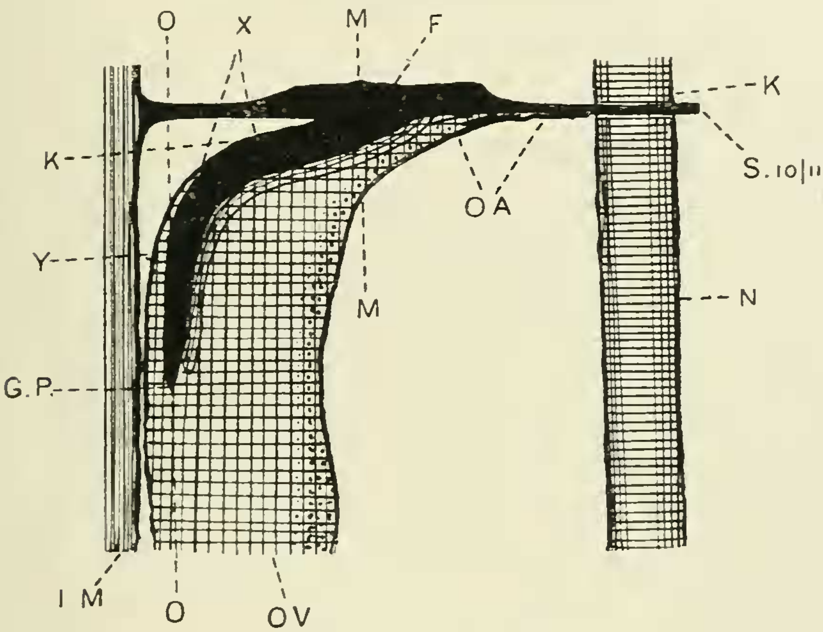
In Tubifex the septa are thin membranes, consisting of a middle layer formed of connective tissue and muscle fibres, and on each side of this layer a covering of cœlomic epithelial cells (Pl. 24, fig. 9, s.). Even in very young worms the septa upon which the ovary and testis are attached are somewhat thicker, and the cœlomic cells more numerous than on other septa.

When the funnel of the male duct is beginning its differentiation, the first sign is found in multiplication of the cœlomic cells on the front of septum 10/11, in a position ventral to a horizontal line drawn through the middle of a transverse section of the gut, but a little above the nerve chord to the right and the left on each side of the body. The ovary in Tubifex is attached near the nerve chord, and hangs upwards in the segment eleven, as is shown in Pl. 24, fig. 9, which is longitudinal. In Text-fig. 1, I have drawn a diagram to illustrate the position and method of attachment of the gonad. The points O.A. mark the ventrally placed stalk of attachment of the ovary quite near the nerve chord N.C. From the points O.A. the stalk slopes rapidly towards the body wall (I.M.), being in reality pushed out in this way by the gut. The main part of the ovary (O.) projects upwards on each side of the gut, as is shown in Pl. 24, fig. 9, which is a section through the points O–O in Text-fig. 1. In Pl. 24 fig. 16 is drawn a slightly oblique transverse section, which illustrates the truth of these remarks. The bottom part of the ovary is seen to be attached at Y quite near the nerve chord (N.C.). In this figure the funnel thickening, which has reached a stage like that of Pl. 24, fig. 1, lies between the nerve chord (N.C.) and the bottom chloragogen cell (CH.), as an examination of the next two sections further forward showed. In Text-fig. 1 the gut occupies a position along the letters M.M., while the chloragogen cells fill some of the remaining parts of the cœlom. It will now be clear that the funnel thickening on septum 10/11 lies almost beneath the gut in a ventral position, and in close connection with the stalk of attachment of the ovary.

At the same time as the front cœlomic layer of septum 10/11 is thickening, a number of cells, also from the front cœlomic epithelium, grow back through the middle layer of the septum into segment eleven (Pl. 24, fig. 1). This backgrowth (B.S.D.) of the cœlomic cells of segment ten appears at the most ventral edge of the funnel thickening, as is shown in Pl. 24, fig. 1, and in fig. 16. An examination of a good series of sections shows that this backgrowing chord pierces the septum 10/11 in different regions in different specimens. In Pl. 24, fig. 1, the backgrowth has appeared on a level just above the stalk of attachment of the ovary (OV.), while in Pl. 24, fig. 16, the backgrowth (B.S.D.) pierces the septum (S.P.) beneath the ovary (OV.). No definite statement can be given as to the region of septum 10/11, through which the early sperm-duct backgrowth will pierce; but it can be said that the latter always appears on the ventral edge of the circular funnel thickening of the septum (Pl. 24, fig. 1). This is rather remarkable, for one might possibly expect the sperm duct to appear in the middle of the early funnel. The stalk of the ovary is always found near the backgrowth, and it is often difficult to be sure that the two are different structures (Pl. 24, fig. 16). Quite close up to the place of origin of both the ovary stalk and the sperm-duct rudiment, it is difficult to distinguish between septal cells, cells of the stalk of the ovary, and backgrowth cells ; and very often in transverse section it is almost impossible to follow the duct through the septum to the funnel thickening in front. This difficulty is never so apparent in longitudinal sections. Invariably the early sperm duct is at first attached to the stalk of the ovary, as is shown in Pl. 24, figs. 1, 16, and diagrammatically in Text-fig. 1. The ovary, then, always provides the first support of the duct. From this position, marked by x and y in Pl. 24, fig. 16, the backgrowth may become directly attached to the body wall, or it may continue growing up the side of the ovary. In Text-fig. 1 the path of an early sperm duct is shown semi-diagrammatically. At the letter M the duct leaves the funnel (F.), runs along the stalk of the ovary at X till it comes into contact with the body wall. It remains partly in contact with the body wall and partly with the ovary at the region X. It then temporarily leaves the body wall, creeping instead up the ovary, which slopes upwards. This part of such a duct is cut in Pl. 24, fig. 9. The duct (S.B.G.) is seen to be mounting upwards, using the ovary as a support. The growing point (XY) is, in reality, on its path towards the body wall, as I have shown in Text-fig. 1. When the duct has reached a position about on a level with a line drawn horizontally through a transverse section of the gut—i.e., about half way up the body wall—the growing point continues on its path backwards parallel to the nerve chord (Text-fig. 1). The reason for this seems to be that the backgrowth appears on the ventral edge of the circular funnel thickening of septum 10/11, because the stalk of the ovary is situated there, and is necessary for the support of the early sperm-duct growth on its path backwards. Were the ovary to be attached higher up, one would expect that the duct would pierce the septum in the new position. In fact, it is the slightly varying position of attachment of the ovary which causes the locality of piercing of the backgrowth to vary. It is only after the backgrowth has left the stalk of the ovary that it begins to mount upwards.

It never seems to grow back *per se*, unattached to any other organ in the cœlom, but always uses either the ovary or the body wall as a support. Even where in sections the back-growth seems to be unconnected to any body, closer examination will show that protoplasmic bridges serve to place the cord in communication with other organs. Pl. 24, fig. 13, shows the rudimentary sperm duct (SP.D.) and its connections with ovary (OV.) and body wall. The growing end of the

TEXT-FIG. 1.



Semi-diagrammatic plan of the early sperm-duct backgrowth viewed from above. At the point X the backgrowth was in contact with the up-curving body wall. At Y the growth has no connection with the cœlomic wall, but the growing point (G.P.) is approaching the side of the body wall. Pl. 24, fig. 9, is drawn from a section between the points O–O. The attachment of the ovary (O.A.) is near the nerve chord (N.C.). This may be compared with Pl. 24, figs. 1 and 16. Pl. 24, fig. 1, is a section through M–M at about this stage. The point X in this Text-figure is identical with the same point X in Pl. 24, fig. 16, where the backgrowth first meets the cœlomic epithelium. This present figure was drawn from a specimen where the duct grows above the ovarian stalk (O.A.). In Pl. 24, fig. 16, the duct pierced the septum 10/11 below the ovary (OV.). (Drawn from partial reconstructions.)

sperm duct is pointed, and consists of a flattened, elongated cell, as is drawn in Pl. 24, fig. 9.

After the duct reaches the cœlomic epithelium of the body wall it is very difficult to distinguish between those cells forming the duct and the cœlomic cells on the walls of segment eleven. It is sometimes possible to notice that the nuclei of the backgrowth stain a little more heavily than the cœlomic nuclei, but I have been unable to make certain whether any cells along the body wall contribute to the duct. From the mode of origin and its early behaviour it seems very likely that the duct is quite independent of the cells of the cœlomic epithelium of segment eleven. Nevertheless, the passing backwards of the growing end of the duct seems to exert some influence on other cells on the body wall, for along the course of the duct the cœlomic nuclei are more numerous than in other regions. This sympathetic activity is specially noticeable towards the distal end of the duct. The back-growing cord penetrates backwards until it reaches a position right above the ventral bundle of chætæ of segment eleven. It now grows down sharply at right angles to its previous path, parallel to the transverse axis of the animal, and penetrates the muscle layers, as is shown in Pl. 24, fig. 14. In fig. 15 the communication between cord and epidermis is complete. Both these figures are drawn from the left side of the section.

Pl. 24, fig. 13, is drawn from the same individual as Pl. 24, fig. 14, the ovary (ov.) in each drawing being in nearly the same position. Fig. 13 was six sections further forward than fig. 14.

In the latter figure it will be noticed that the epidermis has synchronously thickened just where the future male pore will be situated, while the outgrowth of the sperm chord has yet to penetrate the circular muscle layer. It is a curious fact that the sperm duct should grow down from the middle of the body wall at right angles to its previous path. This is shown in a later stage in Pl. 24, fig. 4, on the right side. At the time the sperm duct has connected with the epidermis (Pl. 24, fig. 15), there is no lumen in any part of the duct. The further differentiation of the sperm funnel between the stage of Pl. 24, fig. 1, and the stage when the duct is continuous with the epidermis, will now be described. At an early period the funnel is merely a circular area on the septum, consisting of a group of nuclei (Pl. 24, fig. 1). Not long after the stage when the epidermis and the sperm chord have met, the funnel has reached a stage such as that of Pl. 24, fig. 2, the points x, x of Pl. 24, fig. 1, have grown out to form an edge, while the more centrally-placed nuclei have become arranged with their long axes forwards; the whole cell structure becomes more columnar, especially towards the centre of the funnel. By the time stage drawn in Pl. 24, fig. 2, is reached, some cells from the testis have broken free and are developing in the cœlom (SP.Z.). As has already been pointed out, the duct in Pl. 24, fig. 1, is very short and incomplete, but in fig. 2 it has reached the epidermis, and the distal end has acquired a lumen for a short distance.

At S.D. the sperm duct in Pl. 24, fig. 2, has been cut across on its path towards the body wall (L.M.), and consists in section of about three cells. At the bottom right-hand side of the duct is drawn a small cell, which is flattened upon the duct. The origin of such cells as this will be dealt with later. Pl. 24, fig. 1, also shows that the funnel is essentially derived from the front of septum 10/11, for the middle connective tissue and muscle layer still lies between the two cœlomic epithelial layers, and is only interrupted at the place where the backgrowth has pierced the septum. Pl. 24, fig. 3, shows that the duct (B.S.D.) now is attached to the middle of the funnel—contrast with fig. 2, where the duct has still the same relation to the funnel as drawn in fig. 1. This change of position seems to be brought about partly by the thickening of the duct and the consequent parting of, cells which bind the duct to the ventral edge of the funnel. In this way all other attaching cells become suppressed except near the middle of the funnel. In Pl. 24, fig. 3, the edges XX are still growing outwards, while the inside of the funnel is now profusely ciliated. A short, ingrowing lumen has appeared in the centre of the funnel, but the proximal end of the duct itself is still solid (Pl. 24, fig. 17, B). In Pl. 24, fig. 3, the stalk of attachment of the ovary is shown, the duct appearing to the left of the latter. The subsequent development of the funnel is not different from what has already been described. The edges xx in fig. 3 grow outwards till the funnel is formed, and the whole structure becomes provided with long cilia, especially on the edges. In the adult organ the cells are not so columnar as is shown in fig. 3, nor does the funnel of the adult worm stain so heavily as does the developing organ of a stage such as Pl. 24, fig. 2.

The differentiation of the male duct was described up to a stage when it is merely a solid chord connecting to the epidermis (Pl. 24, figs. 13 and 15). Soon after the connection between the duct and the latter is established, a lumen appears at the most distal end (incipient in Pl. 24, fig. 15), and the rapid growth and lengthening of the organ causes it to break away at places and to begin to form folds in the cœlom. As this process goes on, the whole duct becomes coiled, and, as we know from the adult, soon forces back even into the segment behind. Long before this happens, however, much differentiation has occurred in the duct. In Pl. 24, fig. 17, I have drawn stages in the formation of the proximal part of the sperm duct. In Pl. 24, fig. 13, the latter (S.P.D) is seen to consist of about three cells in a transverse section. Now this primitive duct is found in later stages to become provided with a partial covering of cells (darkened in Pl. 24, fig. 17, A), which in the adult organ forms the epithelial covering of the sperm duct. The origin of these cells is undoubtedly difficult to make out, but I feel convinced that they arise from the cœlomic epithelium of segment eleven. They can first be found on the distal and proximal ends of the duct (see the darkened nucleus on the duct I.S.D. in Pl. 24, fig. 2). Moreover, they appear latest on the middle of the duct. In Pl. 24, fig. 4, these cells have already flattened themselves upon the sperm duct (C.E., C.E.), and it will be noticed that they do not cover that part of the duct which penetrates the muscle layers. The covering cœlomic layer is quite continuous with the cœlomic epithelium of the body wall near the outgrowing duct and at the proximal end near the funnel, and is, I believe, derived from those regions. I do not believe that the median region in the length of the duct receives its covering by proliferation of the cells already forming the chord; but it is quite possible that some cells forming the cœlomic covering of the mid-region of the duct are derived from that part of the cœlomic epithelium of the body wall to which the duct is at first attached. Probably what really occurs in most cases is that the cœlomic cells on the back of septum 10/11 grow back along the surface, and the cœlomic cells in the region near where the duct meets the epidermis grow forward along the surface of the simple duct (Pl. 24, fig. 13) until they meet about mid-way, and the covering is completed. This would account for the fact that the mid-region of the duct is, more often than not, the last to become provided with the cœlomic epithelium.

It has been mentioned already that the lumen first appears in the duct at the extreme distal end. Though the distal end develops latest of all the other parts of the duct, its lumen appears almost immediately after it meets the epidermis. It is broadly true that the lumen of the duct appears from behind forwards, though the early funnel soon acquires a small cavity (Pl. 24, fig. 3).

The stages in the formation of the lumen in the proximal end of the duct are given in Pl. 24, fig. 17. In Stage A the rudimentary duct has already been provided with its covering cells (darkened).

By Stage B the inner core of cells has undergone an important step in differentiation, the cells having become arranged regularly, with their longer axes meeting together in the centre. The cœlomic epithelial covering has also become more definite.

In Stage C the multiplication of all the cells has caused the nuclei to become smaller, and a narrow lumen has appeared. Almost directly the lumen is formed cilia appear in it, and by Stage D the cilia are profuse. This stage shows the incipient striation of the cells forming the duct; this striation is a peculiarity of the adult duct, and has been commented upon by Benham (**4**) in another Tubificid.

In Stage E, which is almost complete, the nuclei are now small, and a striation of the whole duct is pronounced, especially around the lumen. The cœlomic epithelium is much drawn out, and the nuclei are very small. The adult duct is not very much further differentiated from what is drawn in Stage E, and a description of further stages would be unnecessary. The development of the distal end of the sperm duct is necessarily complicated by the later appearance in that region of the cement gland and atrium. Vejdovsky (**5**) has shown that the cement gland develops from the lining epithelium of the distal end of the sperm duct.

The atrium is formed by a thinning out of the distal end of the duct, so as to make a thin-walled sac of larger size than the rest of the duct.

In Pl. 24, fig. 15, the cœlomic cells which grow outwards to meet the epidermis, and those which later form the cœlomic epithelial covering of the duct are not to be distinguished from one another. In Pl. 24, fig. 4, this distinction is quite clear. After this stage one may consult Vejdovsky for the formation of the spermiducal gland and for the atrium.

Before leaving the sperm duct, it might be mentioned that the muscle layer of the lower region of the male duct—*i.e.* that of the atrium—is not found in early stages (Text-fig. 4). It is only some time afterwards, but before the prostate and atrium have appeared, that the muscle layer appears. The origin of this layer is apparently due to the cœlomic epithelium, and it must be the same epithelium that forms the few muscle fibres along the duct itself. These are difficult to see in the adult worm; but in the young the muscle layer of the bottom region of the sperm duct is very thick, and only later becomes thinned out as the atrium is formed.

The Development of the Oviduct.

I have already mentioned that the oviduct is the last organ of the whole genitalia to appear. As is well known, this duct is simply a small funnel opening internally into segment eleven, and externally just where the segments eleven and twelve meet (Pl. 24, fig. 6, x). A little after the sperm-duct funnel has reached a stage such as that of Pl. 24, fig. 3, and when the duct is provided with a perfectly formed lumen towards and at its distal end, the cœlomic epithelial cells on the ventral edge of septum 11/12, and those on the floor of the cœlom of segment eleven nearest the place where the adult oviduct is found, multiply so as to form a close plug of nuclei. Pl. 24, fig. 5, c.p., though drawn from a little later stage, gives a correct impression of the appearance of this plug in early stages. In Pl. 24, fig. 7, the plug is shown in transverse section just after it has begun to grow outwards towards the epidermis; the longitudinal muscle layer has already been pierced. It will be noticed that, at this time, the oviducal rudiment has a very close resemblance to the early stage in the formation of the spermatheca drawn in Pl. 24, fig. 10.

The nuclei of the cœlomic cells at the time the oviduct begins to form are a little smaller than when the sperm duct first appeared. This is made quite apparent by examining and comparing Pl. 24, figs. 7 and 8, with Pl. 24, figs. 1 or 13. Even at the early stages drawn in figs. 7 and 8, the upper cells of the plug have a partly detached and ragged appearance (y). In both figs. 7 and 8 the epidermis is still normal, but not long afterwards the clitellum begins to develop; but the oviduct still remains a solid chord, with neither lumen nor funnel. In stages drawn in Pl. 24, figs. 5 and 6, clitellum is well advanced, and yet no cavity has appeared in the outgrowth.

The true funnel and lumen of the duct apparently only appear a short time before, or when the eggs are ripe; but the solid connection between epidermis and cœlom, of course, is present long before any eggs mature. Even in adult worms one cannot discover a lumen in the oviduct, and one concludes, therefore, that any cavity in the duct is of temporary appearance, and caused by the outpushing of the eggs. The duct is really only a region of the body wall which has been prepared beforehand by the thinning out of the clitellum, and by the internal collection of cells near where the eggs pass through the wall of the body. When one speaks of the "funnel" of the oviduct, one uses a term which gives a wrong impression. I have never found cilia on the oviduct, nor do I think that the ragged inner edge of the outgrowth of cœlomic cells which constitute tbe oviduct is quite aptly described by the term "funnel." Provided that one knows where to look for the oviducal rudiments, one has no difficulty in identifying the earliest stages in the formation of this organ. No muscle is found in connection with the oviduct of Tubifex.

Development of the Spermatheca or Receptaculum Seminis.

Bergh (**1**), in his paper on the development of the genital organs in Lumbricids, describes the spermatheca as an epidermal invagination which pierces both muscle layers and protrudes into the cœlom. His details of the early stages are so meagre that I cannot compare the formation of this organ in Lumbricus with what occurs in Tubifex.

A very short time after the sperm-duct rudiment begins to grow back from septum 10/11 an examination of the mid-ventral region of segment ten will show two thickenings on either side of the body wall. This plug-like aggregation of cells grows out towards the epidermis, as is shown in Pl. 24, fig. 10. In the latter figure the ventralmost nuclei have reached the circular muscle layer. A comparison of this figure with the one drawn in Pl. 24, fig. 7, will show that the early stages in the development of the oviduct and spermatheca are nearly identical, except for the position and time at which they begin differentiation; both begin as outgrowths of cœlomic epithelium. About the time the plug reaches the circular muscle layer the epidermis immediately below thickens synchronously (fig. 10). The cœlomic plug never pierces the circular muscle, but the epidermis now begins to invaginate, carrying the circular muscle layer with it (Pl. 24, fig. 11). The cœlomic cells are pushed aside, and the ingrowth becomes situated in the substance of the plug, which forms a cap over it.

As the invagination grows, it carries the cœlomic cells in with it, as is shown in fig. 12; while the bulk of the early cœlomic thickening forms a region encircling the mid-part of the spermatheca. Little by little, as the spermatheca becomes larger, the cœlomic cells at the point Y become fed out till they form a single layer on the surface of the invagination (fig. 12, S.L.). That part of the spermatheca near the pore becomes, in later stages, drawn out to form a duct, and the whole organ then appears clavate. In the adult, cilia are to be found in the duct leading from the pore to the cavity of the spermatheca. These appear at a stage when the duct is constricted to form a narrow tube. The lower end of the duct never seems to have cilia, only that part of it leading immediately into the swollen terminal portion being so provided, and they are so short as to be easily overlooked. Vejdovsky has described the later stages in the formation of the spermatheca after my fig. 12, but he has overlooked the stages in figs. 10 and 11. Towards the end of differentiation there is another muscle layer added to the circular one which has been derived, as I have already shown, from the circular muscle layer of the body wall. This second and outer layer seems to be formed from the cœlomic epithelium of the spermatheca, which becomes modified in the regions where the muscle is best developed. The fibres run longitudinally.

I would venture to suggest that the cœlomic epithelial outgrowth figured in Pl. 24, fig. 10, has more significance than if it were only a preparatory stage merely aimed at separating the thicker muscle layer of the body wall before invagination commences. If this cœlomic cell plug were to break through the circular muscle layer to the epidermis, it would resemble, in almost every particular except in its position, the oviduct. The stage drawn in Pl. 24, fig. 7, may be compared with Pl. 24, fig. 10. The suggestion naturally occurs that this outgrowth represents the last remains of the genital duct of segment ten, being, in fact, the distal extremity of the original duct. The invagination of the spermatheca may possibly represent the same region near the epidermis of the genital duct of the male segment, the region which in the male duct gives rise to the penis.

A somewhat awkward fact which is difficult to explain if we embrace the view that the spermatheca of the Oligochæta is the remains of the genital duct, is that many spermathecæ may occur in one segment (fourteen in Kynotus madagascariensis), and, moreover, that spermatheca quite often occur in the same segments as the male or female genital duct (see Beddard's monograph). Bergh believed all spermathecæ to be new structures. In the case of Tubifex, one might suppose that the spermatheca was first developed in connection with the orifice of the genital duct, and that the cœlomic epithelial plug is the remains of the old structure, while the epidermal ingrowth is the new. It is almost certain that the numerous spermathecæ in Kynotus are new structures, but it is quite possible that the Tubificid spermatheca is not homologous, but only analogous with the Kynotus spermatheca. It must, however, be admitted that such suggestions as I have brought forward are only within the bounds of pure hypothesis, and that no satisfactory conclusion can be drawn from the scanty evidence we have to work on.

Addendum.

Just as this paper was being finished Miss G. C. Dixon's 'Monograph of Tubifex' (6) came into my hands. Miss Dixon states that she has found spermatozoa of two kinds in the sperm sac. I have examined my sections of adult worms to see if I could find any dimorphism, and I could come to no satisfactory conclusion from sections either of the sperm sac or of the spermatophore. Smears were then made of the sperm sacs, and these were found to be more useful. I have not been able to make sufficient preparations, and I do not wish my words to be taken as my final opinion as regards the dimorphism of the spermatozoa of Tubifex rivulorum but I feel sure that neither of the spermatozoa drawn in Miss Dixon's monograph are mature. The heads of the Tubificid spermatozoa I have found, when drawn at the magnifications given in Miss Dixon's paper, are much longer than her supposedly ripe sperms, and, moreover, much thinner. I have found plenty of spermatozoa of the two sizes drawn by Miss Dixon, but they are not ripe, and intermediate stages are found in plenty.

Miss Dixon says: "If, however, in a mature worm one seeks for the testes, one will not be able to find them"; and in another part "that the latter [testes] soon disappear." In all my preparations of adults, containing ripe eggs and spermatozoa, I have been able to find testes, though they are slightly smaller than when the worm is half grown. Another of Miss Dixon's statements with which I am sorry to disagree is that the testes cannot be found in the adult because "they have been completely enclosed in the sperm sac." As a matter of fact, the sperm sac is not formed from that part of the septum near the nerve chord, alongside which the testes are attached. The position of the latter is not affected in any way by the formation of the sperm sac.

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EXPLANATION OF PLATE 24.

Illustrating J. Bronté Gatenby's paper on " "The Development of the Sperm Duct, Oviduct, and Spermatheca in Tubifex rivulorum."

Lettering.

B.S.D. Backgrowing sperm duct. B.V. Blood-vessel. C.E. Cœlomic epithelium. C.H. Chloragogen cells. C.H.T. Chæta. C.L. Clitellum. C.M. Circular muscle layer. C.P. Cœlomic cell-plug. E.P. Epidermis. G. Gut. L.M. Longitudinal muscle layer. M. Muscle layer. M.P. Male pore. N.C. Nerve chord. OV. Ovary. S.P. Septum. S.D. Sperm duct. S.P.Z. Developing spermatozoa. T. Testis. X, Y, and SL refer to special parts of the figures mentioned in the text.

All figures except Fig. 9 have been drawn by camera lucida with a Zeiss 2 mm. oil immersion, and eye-piece 4. Fig. 9 was drawn with the same oil immersion and compensating eye-piece 8. In reproduction the figures have been reduced by one half.

Fig. 1.—Longitudinal section of a very early stage in the formation of the sperm funnel and duct. The sperm-duct backgrowth (B.S.D.) is seen to leave the septum 10/11 at the bottom of the sperm funnel thickening. This figure is drawn from such a section as that through the points M–M in Text-fig. 1. At XX the cells on the front of the septum 10/11 are multiplying rapidly to form the funnel.

Fig. 2.—Horizontal section of septum 10/11 at a later stage. The funnel is now a noticeable structure, and the points XX are still growing; at these edges of the funnel the nuclei are dividing rapidly, are smaller than those in the centre of the funnel, and are not arranged in a definite manner. The cells forming the more centrally placed part of the funnel have small cilia, and their nuclei have their long axes

pointing forwards. The sperm duct by this time has reached the exterior, and is at a stage somewhat like that drawn in fig. 15. At S.D. the duct is cut transversely, and at B.S.D. the outline of the rest of the duct is dotted in. At S.P.Z. some developing spermatocytes are seen free in the cœlom of segment ten.

Fig. 3.—Horizontal section of the septum 10/11 at a much later stage. The points XX are still growing outwards, but the centre of the funnel is now profusely ciliated. A short, narrow lumen has appeared in the centre of the funnel, but the rest of the proximal end of the duct is still blind, though the more distal end has reached a stage such as that drawn in Fig. 4. The ovarian stalk is cut across to the right of the duct (B.S.D.).

Fig. 4.—Transverse section of male opening (M.P.) and distal extremity of male duct before formation of atrium and penis, and after the appearance of the lumen. To follow after figs. 14 and 15.

Figs. 5 and 6 are consecutive longitudinal sections of the oviducal region of septum 11/12 a good time after the connection between the cœlomic plug (C.P.) and the epidermis has taken place. The clitellum is almost fully formed, but the oviduct is still incomplete. At X in fig. 6 the future oviducal pore will appear.

Fig. 7.—A very early stage in the formation of the oviduct. Transverse section just in front of septum 11/12. The plug of outgrowing cœlomic cells has penetrated the longitudinal muscle layer, but has still to pass the circular layer. The upper end of the plug has the characteristic ragged appearance of the early forming oviduct.

Fig. 8.—A little later stage after the junction of the epidermis (E.P.) with the cœlomic nuclei. The upper end of the young oviduct has the same ragged appearance (Y) as is shown in fig. 7.

Fig. 9.—A highly magnified longitudinal section through the growing point of the early sperm duct. This figure is drawn from such a section as that through the points O–O in Text-fig. 1. The growing point (XY) is seen to consist of a single, much flattened cell. The early duct is mounting upwards, using the ovary as a support. At S. the septum 10/11 is cut, though the thickening of the funnel is more towards the nerve cord. Fig. 1 is drawn from a stage of about the same age as fig. 9.

Fig. 10.—A very early stage in the formation of the spermatheca. A cœlomic cell plug (C.P.) is growing outwards, parting the longitudinal muscle layer. At X the epidermis has synchronously thickened. T= the testis.

Fig. 11.—A later stage in the formation of the spermatheca. The epidermis has begun to invaginate, pushing into the cœlomic cell plug, which forms a sort of cap over the early spermatheca. The circular

muscle layer is still intact, and becomes carried in by the invaginating epidermis, forming one of the muscle layers of the adult spermatheca.

Fig. 12.—A still later stage to show how the remains of the cœlomic cell plug (YY) becomes fed out, eventually forming the single cœlomic epithelial layer of the spermatheca. At S.L. these cells are already one layer in thickness. As the neck of the spermatheca becomes longer, the cells at Y become stretched out to form the covering.

Fig. 13.—Transverse section of the early sperm duct just after it has reached the exterior (fig. 14). The sperm duct is connected to the ovary (OV.) and body wall by elongated cells.

Fig. 14 was drawn from the same specimen as fig. 13, only six sections further back. This shows that the sperm duct grows vertically downwards from its position near the ovary (fig. 13) in order to grow outwards to meet the epidermis. In the transverse section of this region of segment eleven a fairly long part of the sperm duct is cut in the same section (figs. 4, 14, and 15). In fig. 14 the outgrowing cœlomic epithelial cells have yet to pass through the circular muscle layer. At C.H.T. the ventral bundle of chætæ of segment eleven is partly cut across.

Fig. 15.—The cœlomic cells have penetrated the circular muscle layer and have reached the epidermis. A cavity has begun to appear in the scattered cells forming the outgrowth. All the region near this downgrowing sperm duct was much affected by the presence of the latter, for the cœlomic cells had multiplied much more than at any other part of the cœlom of segment eleven.

Fig. 16.—Obliquely transverse section through the septum 10/11 to show early sperm-duct (B.S.D) attachment of ovary to septum and surrounding structures. This figure is drawn from such a section as that through the points K–K in Text-fig. 1. The sperm-duct backgrowth has pierced the septum beneath the place of attachment of the ovary (compare with figs. 1, 9, and Text-fig. 1). The cells marked C.H. are chloragogen cells belonging to segment eleven, but on the right-hand side of the figure the section cuts through the septum 10/11.

Fig. 17.—A B C D and E. Stages in the formation of the proximal end of the duct.

The nuclei marked more darkly in A will form the cœlomic layer of the sperm duct. At stage C small cilia have appeared. By stage E some muscle fibres (M.) can be found on the outer wall of the duct, and the cells forming the latter have become peculiarly striated.